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Article

The Evaluation of Microplastic Reduction in Biofloc Aquaculture for Sustainable Nile Tilapia Cultivation

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Abstract. Sustainable aquaculture requires environments free from microplastic contamination. However, microplastics are now commonly found in aquatic systems, including fish farms, where they can accumulate in organisms and enter the food chain. This study evaluates the effectiveness of biofloc technology in reducing microplastic levels in water and Nile tilapia (*Oreochromis niloticus*), using ecological risk indices: Pollution Hazard Index (PHI), Pollution Load Index (PLI), and Potential Ecological Risk Index (PERI). The experiment lasted 50 days with four treatments, including polyethylene (PE) and polyethylene terephthalate (PET) exposure. Microplastic concentrations ranged from 0.12 to 0.33 particles/L, with highest accumulation in the fish esophagus (39.2 ± 6.87 particles/g). Identified polymers included PE, PVC, and PA. Risk indices showed PHI = 166.69, PLI = 1.01–1.66, and PERI = 21.49, indicating medium to high ecological risks. Results show that biofloc effectively reduces microplastic levels, making it a promising solution for sustainable aquaculture. The study highlights the need for better plastic waste management policies and stricter regulation of PVC and PET near farming areas.

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1. Introduction

Aquaculture, as a major sector in fulfilling global animal protein needs, faces challenges from plastic pollution, particularly microplastics (MP), which are particles smaller than 5 mm. Microplastics are now detected in almost all aquatic ecosystems, including aquaculture systems, where they accumulate in organisms such as fish, shrimp, and shellfish [1]. Studies have shown that the presence of microplastics not only contaminates water but can also enter the food chain, impacting human health and ecosystems [2].

Biofloc technology is used in aquaculture to utilize microorganisms in processing organic waste, particularly ammonia and nitrate produced by fish or shrimp. In biofloc systems, microorganisms like bacteria, algae, and fungi thrive in water rich in dissolved organic matter [3-4]. This process converts organic materials into microbial biomass, which can be used as supplementary feed for fish or shrimp. Biofloc helps maintain water quality by reducing organic matter buildup and lowering toxicity levels in the culture pond [5-6].

While biofloc is well-known for its ability to reduce organic pollution and improve water quality, it also has the potential to accumulate microplastics [7-8]. These microplastics may originate from water contamination or waste inadvertently introduced into aquaculture systems. Research by Yuan, Nag, and Cummins (2022) [9] indicated that although microplastic concentrations in biofloc are typically lower than in surrounding water, biofloc can reduce microplastic levels in the pond by adsorbing these particles.

Few studies have explored biofloc's role in reducing microplastics in aquaculture, but some suggest that biofloc microorganisms, like bacteria and fungi, can break down and adsorb microplastics. For instance, bacteria such as *Bacillus* spp. and *Pseudomonas* spp. have been shown to degrade plastics under certain conditions, though the efficiency varies depending on the type of plastic [10]. Biofloc, with its diverse microorganisms, facilitates the adsorption of larger microplastic particles into the biofloc matrix, which can then either degrade or be trapped in the system.

Quantitative data supports the theory that biofloc can reduce microplastic levels in aquaculture systems. Makhdoumi, Hossini, and Pirsaheb (2023) [11] found that in a biofloc system used for Nile tilapia (*Oreochromis niloticus*), microplastic levels in water decreased by up to 30% after two weeks. This indicates that biofloc not only serves as a medium for processing organic waste but also contributes to microplastic reduction through adsorption and fixation of particles. Makhdoumi, Hossini, and Pirsaheb (2023) [12] also reported a reduction of 25-40% in microplastic concentrations after two weeks in a well-managed biofloc system.

Although biofloc can reduce microplastic concentrations, the presence of microplastics within biofloc poses health risks to the cultured fish. Fish may accumulate microplastics by directly ingesting them from the water or by consuming biofloc as feed. In biofloc aquaculture, fish are at risk of ingesting microplastics trapped in the biofloc, which could affect their digestive systems. Research by [13] found that ingested microplastics disrupt fish digestion, leading to reduced growth and feed efficiency. Studies by [14] also show significant microplastic accumulation in fish tissues, with levels varying depending on the type of plastic and exposure time. Microplastic accumulation can reach 15-30 particles per gram of fish body weight, influenced by fish density and water quality in the biofloc system [15].

Despite these risks, biofloc has potential benefits for improving water quality and reducing broader exposure to microplastics in aquaculture systems. One approach to mitigating these risks is to enhance biofloc management, including regular monitoring of microplastic levels in both the biofloc and water, and reducing plastic pollution sources around aquaculture facilities.

To evaluate the ecological risks of microplastics in aquaculture, indices such as the Pollution Hazard Index (PHI), Pollution Load Index (PLI), and Potential Ecological Risk Index (PERI) have been developed. These indices measure the level of harm and potential ecological risks posed by microplastics in aquaculture waters [15] .Using these indices can help assess the accumulation of microplastics in biofloc systems and their impact on ecosystem health and aquaculture sustainability.

A study by Sharma and Kaushik (2021) [16] demonstrated that PHI and PERI indices could be used to evaluate the risks of microplastics in biofloc aquaculture systems by considering microplastic concentrations in water and fish bodies. Their research found that increasing microplastic levels in the water raised the health risks to fish, with PHI values rising as microplastic concentrations in biofloc increased.

However, to date, there have been few studies that simultaneously evaluate microplastic concentrations in water and fish tissues quantitatively, as well as calculate risk indices in an integrated manner within a biofloc system for tilapia aquaculture. This study is among the first to comprehensively examine the relationship between microplastic concentrations in water, their accumulation in fish tissues, and the calculation of PHI, PLI, and PERI indices as ecological risk parameters in a biofloc system.

This study aims to evaluate the abundance of microplastics in water and fish bodies in biofloc systems, while calculating PHI, PLI, and PERI indices to assess the potential ecological and health risks of microplastics in Nile tilapia aquaculture. The results are expected to provide valuable insights into the relationship between biofloc technology and sustainable, microplastic-free aquaculture.

2. Experimental Section

2.1. Research Procedure

This study was conducted using Nile tilapia (*O. niloticus*) cultured in glass aquariums measuring 75 x 50 x 50 cm^3, with a water capacity of 150 liters. Prior to use, the aquariums were cleaned with a chlorine solution of 50 mg/L to remove dirt and odors, followed by rinsing with running water until thoroughly cleaned and dried. The aeration system utilized PVC pipes with a diameter of 1/2 inch, measuring 100 cm and 60 cm, connected to form a "T" configuration. The vertical pipe is connected to the aerator, while the horizontal pipe distributes the air. The aeration hose at the bottom of the aquarium is equipped with aeration stones to produce air bubbles. Routine checks were conducted daily to ensure the aeration stones were not clogged.

2.2. Biofloc Application

Once the water pH reached 8, molasses was added as a carbon source at a dosage of 100 ml/m^3 or 15 ml per aquarium. After 30 minutes, a probiotic containing biofloc-forming bacteria was introduced at a dose of 10 g/m^3 or 1.5 g per aquarium. The biofloc formation process lasted for 8 days, with the addition of crushed pellets at 1% of the fish's body weight on day five to accelerate floc formation. Water quality parameters, including ammonia, nitrite, nitrate, pH, and dissolved oxygen, were monitored regularly. Floc density was measured by taking 1000 mL of water from the aquarium, placing it in an Imhoff cone, and measuring the volume of floc sediment after 20 minutes. The floc volume was calculated using the formula from Deswati et al. (2023) [17]:

$$\text{Floc volume } \left(\frac{mL}{L}\right) = \frac{Sediment\ volume}{Sample\ volume} \text{ x } 1000$$

2.3. Measured Impact Parameters

This study involved four treatments: A (without biofloc and microplastics), B (with biofloc and without microplastics), C (biofloc with the addition of PE microplastics 80 items/L and PET 800 items/L), and D (biofloc with the addition of PE microplastics 800 items/L and PET 800 items/L). The parameters analyzed included the abundance of polyethylene (PE) and polyethylene terephthalate (PET) microplastics in water and fish bodies, as well as the potential ecological and health risks associated with microplastics in Nile tilapia aquaculture. The assessment was conducted using the Pollution Hazard Index (PHI), Pollution Load Index (PLI), and Potential Ecological Risk Index (PERI).

PLI is used to assess the overall pollution level at a given location by comparing the concentration of pollutants to a baseline or threshold value. The PLI formula is calculated as follows:

$$PLI = (C_{F1} \times C_{F2} \times C_{F3} \times \dots \times C_{Fn})^{1/n}$$

Where CF = C_i/C_0, with C_i representing the detected pollutant concentration and C_0 being the threshold or baseline value of that pollutant. A PLI value > 1 indicates pollution, while PLI < 1 suggests a relatively clean environmental condition [18].

The Polymer Hazard Index (PHI) was developed to evaluate the ecological risks posed by various microplastic polymers in aquatic ecosystems. This index takes into account parameters such as polymer persistence, bioaccumulation potential, and toxicity to aquatic organisms. PHI is calculated based on the contribution of each polymer in the water using a formula that considers the characteristics of the polymers and their concentrations in the environment.

$$PHI=\sum_{i=1}^{n}(C_i \times H_i)$$

Where C_i is the concentration of polymer i and H_i is the hazard factor of that polymer [19].

The Potential Ecological Risk Index (PERI) focuses more on the ecological risks posed by heavy metal pollutants to aquatic ecosystems. This method integrates the toxicity and bioaccumulation potential of each heavy metal in aquatic environments. The PERI formula is expressed as follows:

$$E_r^i=T_r^i \times C_F^i$$

$$RI=\sum_{i=1}^{n}E_r^i$$

Where E_r^i is the ecological risk index of heavy metal i, T_r^i is the toxicity response factor of the heavy metal, and C_F^i is the contamination factor of the heavy metal based on the detected concentration compared to the reference value [20]. A RI value >600 indicates a very high ecological risk, while an RI value <150 indicates a low risk [18].

2.3. Data Processing Design

Statistical tests were performed using one-way analysis of variance (ANOVA) at a 95% significance level ($\alpha = 0.05$) to examine the differences between treatments. Differences between treatments were considered significant if the p-value < 0.05, followed by Duncan's test as a post-hoc analysis. All statistical tests were conducted using IBM SPSS Statistics software version 23. This approach aims to provide a clearer picture of the effects of the treatments tested in this study. Figure 1 illustrates the stages of the research process.

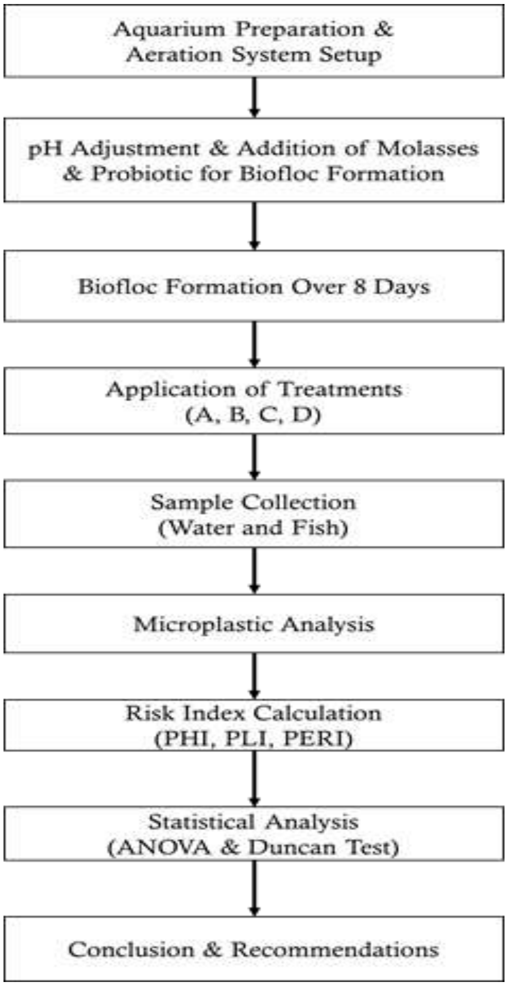

Figure 1. Flowchart of the Experimental Procedure for Assessing Microplastic Accumulation and Risk Indices in a Biofloc-Based Tilapia Aquaculture System

3. Results and Discussion

3.1. Microplastic Abundance Analysis

3.1.1. Microplastics in Water

Figure 2a shows the total microplastic abundance across various treatments in Nile tilapia farming. Treatment A, which lacked both biofloc and microplastics, exhibited 0.17 ± 0.016 particles/L, while treatment B, which included biofloc but no microplastics, recorded a significantly lower value of 0.056 ± 0.012 particles/L. This reduction can be attributed to the activity of biofloc bacteria, which are known to break down microplastic molecules into simpler compounds. These bacteria adhere to the surface of microplastics, forming a biofilm. Within this biofilm, extracellular enzymes produced by the bacteria facilitate the degradation of the chemical bonds in the microplastic polymers through processes like oxidation and hydrolysis, converting them into simpler monomers or oligomers [21].

In contrast, the highest microplastic abundance was recorded in treatment C, with 0.4 ± 0.02 particles/L, followed by treatment D at 0.24 ± 0.087 particles/L. The difference in microplastic concentration between treatments can be attributed to the varying levels of polyethylene (PE) and polyethylene terephthalate (PET) microplastics introduced to test their effects on Nile tilapia farming. The increased abundance in treatments C and D highlights the impact of different plastic types on microplastic levels in aquaculture systems.

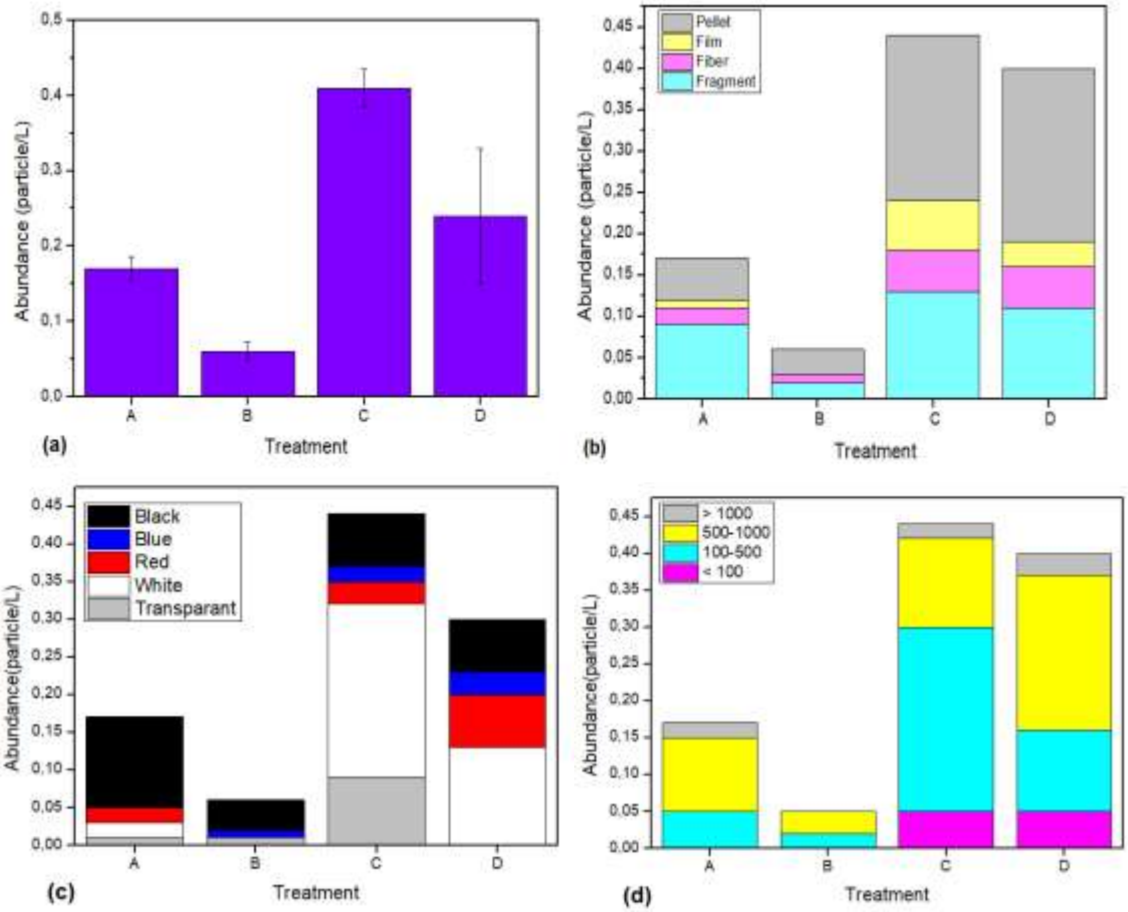

Figure 2. Microplastic abundance in water: (a) Total abundance (particles/L), (b) By shape (particles/L), (c) By color (particles/L), (d) By size (particles/L).

Figure 2b demonstrates that fragments were the most prevalent microplastic shape across all treatments, followed by pellets, fibers, and films. The diversity in shapes reflects the various sources of microplastics, including plastic waste in water, salt production, and direct exposure to PE and PET microplastics. Fragments were found in all treatments, with treatment A having 0.09 particles/L, treatment B 0.02 particles/L, treatment C 0.13 particles/L, and treatment D 0.11 particles/L. Pellets were most abundant in treatments C and D, with concentrations of 0.2 and 0.21 particles/L, respectively. This variety in microplastic shapes emphasizes the different types of plastic waste that contribute to contamination in aquatic environments.

Fragments, the most common type of microplastic, generally originate from the degradation of durable plastic products such as beverage bottles and large plastic containers [22]. Blackburn (2022) [23] identified fragments as one of the most common forms of microplastics found in marine ecosystems, particularly from polyethylene and polypropylene degradation. Pellets, lightweight and easy to transport by wind and water currents, are frequently found along shorelines, beaches, and estuaries [24]. Microplastic fibers, typically shed from synthetic clothing materials such as polyester and nylon, are another significant contributor to aquatic pollution [25]. Finally, plastic films result from the breakdown of thin plastic sheets that are exposed to environmental factors such as sunlight, wind, and mechanical wear. The findings underline the various sources and types of microplastics in aquatic environments and their implications for aquaculture systems. The role of biofloc bacteria in reducing microplastic abundance highlights the potential for biofloc technology to mitigate plastic contamination in fish farming.

Figure 2c shows noticeable variation in the color of the microplastics found, with white and black being the most predominant colors. Treatment C recorded the highest levels of both white and black microplastics, each at 0.23 particles/L. In comparison, treatment A showed a lower abundance of white microplastics at 0.12 particles/L. The white microplastics were identified as pure polyethylene (PE), which were released during the Nile tilapia biofloc-based aquaculture process. These microplastics originated from PE that was not ingested by the fish and remained undegraded by the biofloc bacteria within the system. This suggests that while microplastics are introduced into aquaculture environments, not all of them are broken down or incorporated into the organisms, revealing a limitation of biofloc systems in handling microplastic pollution.

The variety of microplastic colors observed is likely due to the different materials and equipment involved in the aquaculture process, including salt, fish feed pellets, fish nets, and aeration hoses, which are often exposed to microplastic particles. This implies that microplastics enter the aquaculture system through multiple channels, not only from direct exposure to materials used but also from surrounding environmental contamination [26]. Therefore, addressing microplastic pollution in aquaculture requires a more comprehensive strategy that tackles both internal and external sources of contamination.

Figure 2d shows that microplastics within the size range of 100–500 µm were the most commonly found, with the highest abundance recorded at 0.25 particles/L. This finding is consistent with research by Hu et al. (2023) [27], who identified the 500–1000 µm range as the most frequently encountered in aquatic ecosystems. Smaller microplastics, such as those observed in this study, have a higher surface-to-volume ratio, making them more susceptible to colonization by microorganisms that form biofilms. This increases the potential for microplastics to engage with microorganisms that contribute to their degradation process.

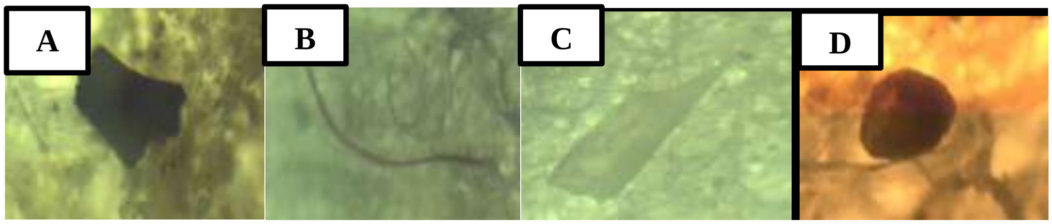

Figure 3. Identification of Microplastics by Shape at 100x Magnification: (a) Fragment, (b) Fiber, (c) Film, (d) Pellet

Additionally, the formation of biofilms on microplastics has significant implications for the accumulation and degradation dynamics of microplastics in aquaculture systems. As noted by Luo et al. (2022) [28], microplastics with larger surface areas are more prone to microbial colonization, including bacteria that aid in the breakdown of microplastic polymers. Therefore, microplastics in the 100–500 µm size range may play a crucial role in the interactions between microplastics, aquatic organisms, and the surrounding environment, influencing how microplastics accumulate and degrade in aquaculture systems.

3.1.2. Microplastics in Fish

Figure 4a demonstrates that Treatment A shows the highest levels of microplastic accumulation across several organs, with 8.08 ± 0.15 particles/g in the intestine, 10.4 ± 0.12 particles/g in the esophagus, 4.96 ± 0.14 particles/g in the gills, and 3.86 ± 0.28 particles/g in the flesh. This indicates that microplastics are readily ingested and retained in various parts of the fish's digestive system. On the other hand, Treatment B shows a significant reduction in microplastic accumulation, with the intestine containing 4.04 ± 0.087 particles/g, the esophagus 4.05 ± 0.061 particles/g, the gills 0.85 ± 0.058 particles/g, and the flesh 0.85 ± 0.09 particles/g. These findings suggest that biofloc bacteria might play a role in reducing microplastic contamination, potentially due to their capacity to break down microplastics, as supported by Yu et al. (2023) [29], who noted that heterotrophic bacteria in biofloc systems can degrade microplastics, thereby minimizing their accumulation in fish tissues.

In Treatment C, microplastics are primarily found in the gills and esophagus, with concentrations of 11.98 ± 0.15 particles/g and 37.8 ± 1.4 particles/g, respectively. This suggests that these organs, particularly the esophagus, are more susceptible to microplastic accumulation. In Treatment D, the esophagus of Nile tilapia (*O. niloticus*) contains the highest microplastic load at 39.2 ± 6.87 particles/g. The esophagus's narrow structure and the mucus lining it likely contribute to the retention of microplastics. The mucus acts as an adhesive, trapping microplastic particles and preventing their passage into the stomach or intestines. According to [30], the esophagus is a key site for microplastic accumulation because it serves as a transitional organ where particles have ample time to adhere to the mucus, increasing their likelihood of being retained.

These findings emphasize the importance of considering the interaction between fish physiology, biofloc bacteria, and microplastic retention when designing aquaculture systems. They also highlight the potential of biofloc technology to mitigate microplastic contamination and contribute to the development of more sustainable aquaculture practices.

Figure 4b illustrates the prevalence of different microplastic shapes, with fragments being the most common, followed by fibers, pellets, and films. Fragments are often ingested by fish because their irregular shape closely resembles natural prey like zooplankton and small invertebrates. This resemblance increases the risk of unintentional consumption, which can cause physical harm to marine organisms and release harmful chemicals as the plastic degrades [31]. Fibers, which rank second in abundance, mainly originate from the degradation of textiles and fishing nets. These fibers are typically shed during washing or from the breakdown of fishing gear, and their small size allows them to disperse widely across marine environments [32].

Films, resulting from the degradation of plastic bags and packaging materials, are also commonly found in aquatic ecosystems. As plastic bags break down, they fragment into thin, flat pieces, which are easily ingested by marine life, disrupting feeding behavior and potentially introducing toxic substances into the food chain [32]. The variety of microplastic shapes highlights the complexity of plastic pollution in marine environments, emphasizing the need for immediate action to reduce plastic waste and minimize its harmful impact on aquatic organisms and the broader ecosystem [33].

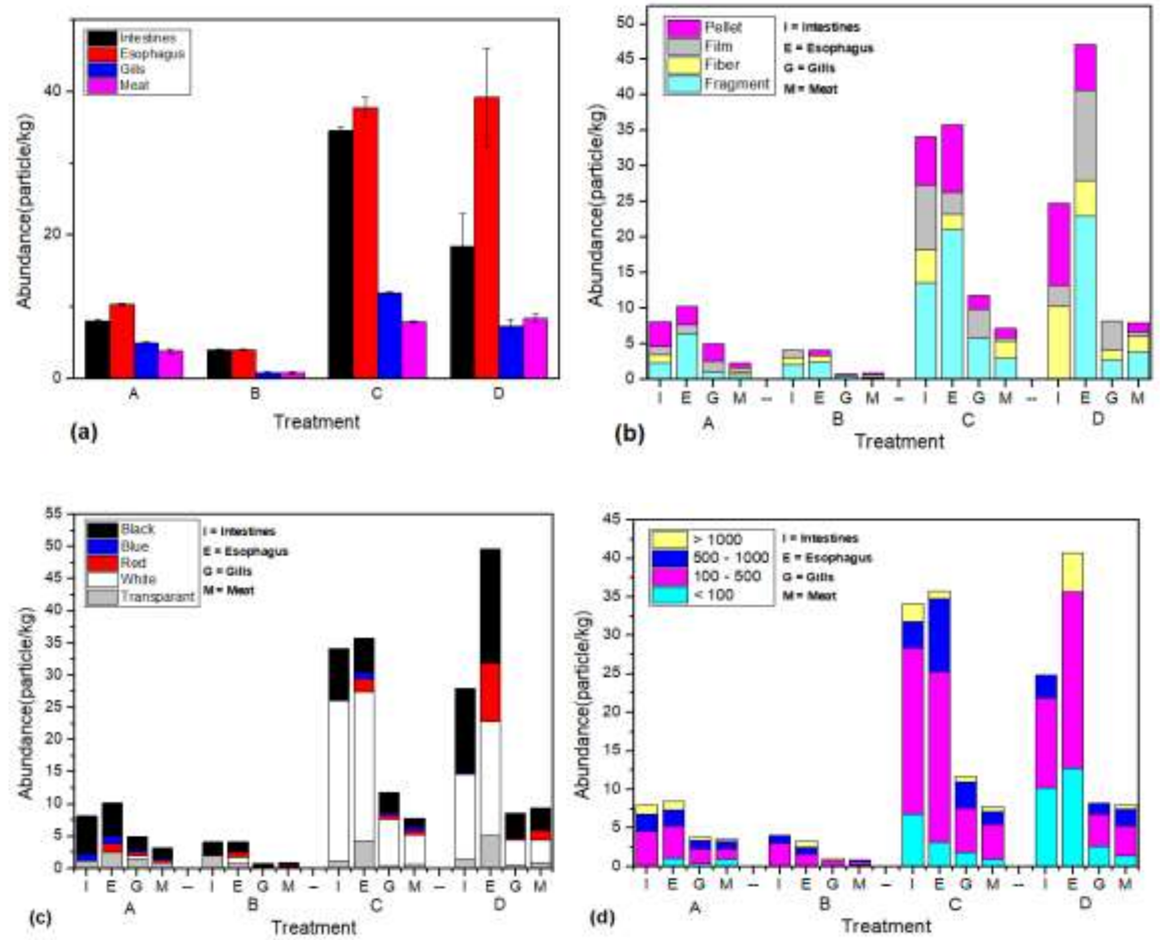

Figure 4. Microplastic Abundance in Fish: (a) Total Abundance (particles/kg), (b) Abundance by Shape (particles/kg), (c) Abundance by Color (particles/kg), (d) Abundance by Size (particles/kg).

In Figure 4c, white microplastics are shown to be the most dominant in terms of color, with a total abundance of 17.82 particles/g. This suggests that the majority of accumulated microplastics are derived from pure polyethylene, a common material used in various consumer products. The prevalence of white particles aligns with findings from other studies where polyethylene, known for its widespread use in packaging and plastic products, is frequently detected in marine environments [33].

Moreover, black microplastics, with an abundance of 15.27 particles/g, are also prominently observed. The presence of black particles in aquatic ecosystems has been linked to their similarity to certain natural food sources, making them more likely to be ingested by aquatic organisms. Fish are particularly susceptible to mistaking these particles for food, as shown in studies that highlight the potential for microplastics to enter the food chain [2]. This unintentional consumption can have significant ecological implications, especially considering the long-term persistence of these materials in the environment.

Figure 4d further illustrates that microplastics within the 100–500 µm size range are the most abundant. These particles are of particular concern because they are small enough to be ingested by fish but large enough to resemble the size of natural prey, such as zooplankton and small invertebrates.

Research indicates that microplastics within this size range are more readily consumed by aquatic organisms due to their physical similarity to aquatic microorganisms [31]. This unintentional ingestion can result in harmful effects on the health of marine species, ranging from physical damage to internal organs to potential toxicity from chemicals leaching from the plastic particles [13].

3.3. Characterization with ATR-FTIR

Figure 5 shows the FTIR spectra of polyethylene (PE) microplastics from three different sources: (a) a white fragment sample from water, (b) a white fragment sample from fish, and (c) a black fragment sample from fish. The spectra reveal distinct peaks for CH_2 stretching in all samples, with prominent peaks observed between 2912.46 cm^{-1} and 2912.91 cm^{-1}, typical of the alkyl chain structure in PE. Both Sample A (white fragment from water) and Sample B (white fragment from fish) show similar peaks at 2912.46 cm^{-1} and 2912.91 cm^{-1}, suggesting the presence of polyethylene microplastics. Sample C (black fragment from fish) also exhibits a comparable CH_2 stretching band at 2912.54 cm^{-1}, confirming the presence of polyethylene. Additionally, the spectra display CH_2 bending around 1464 cm^{-1} and CH_2 rocking near 719 cm^{-1}, which are characteristic of polyethylene, further validating the identification of PE in all the microplastic samples. These findings highlight the widespread occurrence of polyethylene microplastics in different environmental sources and their potential contribution to plastic contamination in aquatic ecosystems [34].

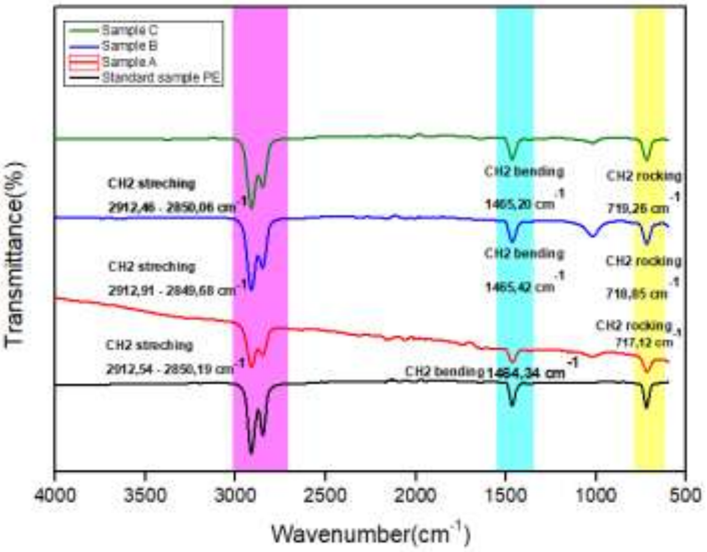

Figure 5. FTIR Spectra of Polyethylene (PE) Microplastics (a) from White Fragment Sample in Water, (b) from White Fragment Sample in Fish, (c) from Black Fragment Sample in Fish.

Figure 6 illustrates the IR spectra of microplastics in two forms: (a) fiber form from fish and (b) polyamide (PA) standard. The spectrum of the fish sample (a) reveals characteristic peaks, including a strong band at 3450.38 cm^{-1}, indicating C-H stretching, and a peak at 2927.97 cm^{-1}, associated with C-H stretching in alkyl groups. Additionally, the peak at 2008.59 cm^{-1} suggests the presence of a carbon-nitrogen bond, and the peak at 1038 cm^{-1} corresponds to C-N bending, indicating nitrogen-containing groups, similar to those found in polyamide. When compared to the polyamide standard (b), which shows similar peaks, this suggests that the microplastics found in fish may be derived from polyamide fibers. These findings point to the potential contamination of fish by polyamide microplastics, which may be a result of environmental pollution from plastic waste, particularly from textile sources [35].

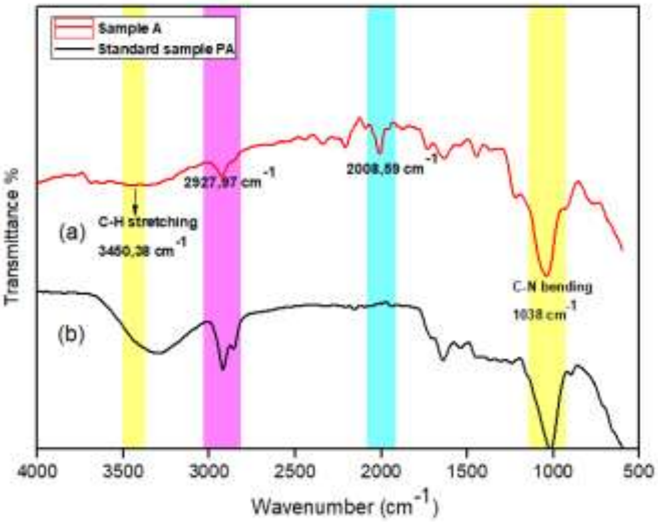

Figure 6. IR Spectrum for Microplastics (a) Fiber Form from Fish and (b) Polyamide Standard.

Figure 7 presents the FTIR spectra of microplastics: (a) black pellet shape from water (Sample A) and (b) polyvinyl chloride (PVC) standard. The spectrum for Sample A (black pellet from water) displays a prominent peak at 2912 cm^{-1}, corresponding to CH_2 stretching, which is characteristic of alkyl chains commonly found in polymer structures. Additionally, a peak at 2206.60 cm^{-1} is observed, likely associated with C≡C stretching, indicating the presence of unsaturation in the polymer. Another notable feature of the spectrum is the peak between 1100-1000 cm^{-1}, suggesting C-C stretching, typical for PVC and other polymer materials. When compared to the standard PVC sample, the spectra show similarities, particularly in the CH_2 stretching and C-C stretching regions, confirming that the microplastic pellet (Sample A) from the water is likely PVC. This indicates that the black pellet microplastics found in the water are predominantly composed of polyvinyl chloride, highlighting its presence and persistence in the aquatic environment [36].

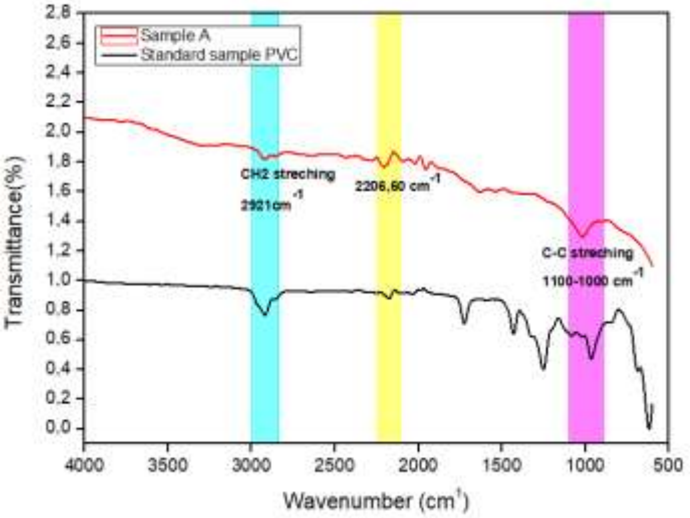

Figure 7. FTIR spectrum of microplastics: (a) Black pellet shape from water and (b) PVC (Polyvinyl Chloride) standard.

The FTIR analysis, as discussed above, shows that microplastics found in aquatic environments and aquatic organisms are composed of different polymer types, such as polyethylene (PE), polyamide (PA), and polyvinyl chloride (PVC). The presence of microplastics in both water and fish samples highlights the extensive pollution of plastic in aquatic ecosystems, potentially harming ecosystem health and aquatic life. By identifying these polymers through FTIR, this study offers crucial insights into the distribution of microplastics and emphasizes the need for effective measures to mitigate plastic pollution in aquatic habitats.

3.3. Risk Index Values and Categories

Table 1 presents the Risk Index values for Pollution Load Index (PLI), Pollution Hazard Index (PHI), and Potential Ecological Risk Index (PERI), which are used to assess the level of microplastic pollution in an aquatic environment. Based on the data obtained in the Current Study, the recorded PHI value is 166.6989, PLI ranges from 1.011 to 1.658, an d PERI is 21.4912, indicating that the environmental condition is categorized under Medium to High-risk levels (II-III), with fluctuating risk levels from Low to Danger.

Table 1. Risk Index Values for Pollution Load Index (PLI), Pollution Hazard Index (PHI), and Potential Ecological Risk Index (PERI).

Risk Index Category	PHI	PLI	PERI	Risk Level
I (Low)	0-1	< 10	< 150	Low
II (Medium)	10-Jan	-	150 - 300	Medium
III (High)	10 – 100	10 – 20	300 - 600	High
IV (Danger)	100 – 1000	20 – 30	600 - 1200	Danger
V (Extreme Danger)	>1000	>30	>1200	Extreme Danger
Current Study	166.6989	1.011 - 1.658	21.4912	II-III, Low to Danger

Microplastic pollution detected in this study indicates significant contamination in the aquatic environment, likely associated with aquaculture systems or other ecosystems. The analysis revealed the presence of polyethylene (PE), polyamide (PA), and polyvinyl chloride (PVC), each with distinct spectroscopic features. PE microplastics, identified by specific C-H stretching and CH bending vibrations, are widely distributed in the environment and have long-term impacts on aquatic ecosystems, particularly by disrupting the food chain (Wang et al., 2023). PA, characterized by unique absorption peaks, is commonly linked to fishing gear and food packaging. Due to its durability, PA microplastics persist in aquatic environments, causing environmental hazards and health risks such as eye irritation, skin issues, and respiratory problems [19].

PVC, a highly durable polymer, was also identified in the samples, with characteristic absorption at 2921 cm^{-1} (CH stretching) and 1100–1000 cm^{-1} (C-C stretching) in the FTIR spectrum. PVC's widespread use in pipes and household products explains its presence in the environment, where it persists due to its resistance to both mechanical and biological degradation. This exceptional durability allows PVC microplastics to contaminate aquatic ecosystems for extended periods [37]. The study highlights the significant environmental and public health challenges posed by microplastic pollution and emphasizes the need for further research and strategies to mitigate its impact.

Higher PERI and PLI values in this study indicate that the studied waters are at high-risk conditions, which could lead to disruption in ecosystem balance. Microplastic pollution, especially PVC, can have adverse effects on exposed aquatic organisms, either through accumulation in their bodies or interactions with other environmental components. For instance, microplastics can serve as carriers for hazardous chemicals (such as heavy metals and organic chemicals) that may accumulate in aquatic biota and enter the food chain, thus posing risks to human health [1]. Therefore, it is crucial to identify the sources of microplastic pollution more specifically and mitigate its impacts on ecosystems and humans.

Further studies are necessary to explore the potential sources of microplastic contamination in broader aquatic ecosystems, as well as to identify effective mitigation strategies to reduce microplastic accumulation in the environment. Some potential approaches to consider include the implementation of more effective water treatment technologies, such as the use of biosorbents or biofiltration, as well as the enforcement of stricter plastic waste management policies, particularly regarding PVC-based products that are frequently found in aquatic environments.

4. Conclusion

This study demonstrates that biofloc technology can effectively reduce microplastic contamination in aquaculture systems, particularly in Nile tilapia (*Oreochromis niloticus*) farming. The dominant microplastics found were fragments and fibers, mostly in the size range of 100–500 μm, with the highest accumulation observed in the fish esophagus. Risk indices such as the Pollution Hazard Index (PHI = 166.69), Pollution Load Index (PLI = 1.01–1.66), and Potential Ecological Risk Index (PERI = 21.49) indicated varying levels of ecological risk, from low to hazardous.

These findings highlight the environmental and health implications of microplastic pollution and emphasize the importance of adopting biofloc as an eco-friendly solution. In response to reviewer comments, the conclusion has been revised to be more concise and reflective of all key findings, including microplastic characteristics and accumulation sites. Additionally, this study now explicitly discusses its contributions to sustainable aquaculture practices and environmental policy, recommending stricter regulation of PVC and PET materials near farming areas. The research also fills a gap by integrating quantitative analysis of microplastics in both water and fish tissues with ecological risk assessment tools tailored for aquaculture environments.

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